10/069561
JC13 Rec'd PCT/PTO 22 OCT 2001

2|prts

SOLUBLE TRAUMA-HEALING HEMOSTATIC CELLULOSE CONTAINING COAGULATION PROTEIN AND METHOD OF PREPARATION THEREOF

Backgrounds of the Invention

1.Field of the Invention

The invention according to the present application relates to soluble trauma-healing blood-stilling cellulose containing coagulation protein and a method of preparation thereof, and more specifically to an internally absorbing soluble wound-covering blood-stilling material promoting blood coagulation and vascular dilation on application to the internal or external lesion and a method of preparation thereof.

2.Description of the Related Art

In the past there types of traumato-hemostatic agent, namely cellulose oxide, gelatin and micro-fibrous collagen, have been known and have already been used as medical preparations and for medical instruments. Cellulose oxide agents achieve their hemostatic action in that the anhydrous polygluconic acid has an extremely strong affinity to hemoglobin and forms a salt with it. This hemo-coagulation promoting effect is due not so much to the agent's action on the blood's coagulation mechanism but rather to physical action. That is to say, that this agent promotes the formation of blood coagulates as the result of the perfusion of blood causing this agent to swell with the development of brown or black gelatin clots. The agent thus displays an ancillary localized hemostatic action and is absorbed in approximately two weeks. Furthermore, microfibrous collagen contains as the main component natural collagen extracted from the bovine dermis and achieves a hemostatic action through platelet agglutination on contact with blood.

Since the cellulose oxide agent does not directly act on the blood coagulation system it has a weak coagulating effect and thus requires approximately two weeks to be completely absorbed in the system so that it acts as an inflammation and adhesion promoting factor in the affected area during this period. Gelatin agents have a slower

absorption than cellulose oxide and are animal derived materials so that there is a considerable possibility of their causing various infectious diseases, including mad cows disease and Kreutzfeld Jakob disease. Moreover, microfibrous collagen takes about a month or longer to be completely absorbed so that it is liable to cause inflammation and adhesion in the affected part during this period. Since the agent is of bovine origin there it carries a risk of infectious diseases such as mad cows disease or or other infections due to unknown viruses.

Hemostasis with the conventional methods using the above hemostatic agents is apt to lead to inflammation and adhesion due to the poor internal absorption of these agents and carries a risk of disease due to unknown infections.

Furthermore, the Japanese Patent Laid-Open No. Hei10-77571 refers to a soluble cellulose fiber whose hydroxyl groups in the glucose units constituting the cellulose molecules of the natural or regenerated cellulose fibers have been partially carboxymethylated so that its etherification degree becomes 1.0% or higher and which exhibits a hemostatic effect by covering the trauma by rapidly dissolving on contact with blood and congealing to a gelatin with the blood.

When the degree of replacement of the carboxymethyl radicals (etherification degree) equals or exceeds 1.0%, however, the agent will not rapidly dissolve even when coming in contact with blood and a large amount of unusable material will remain without exhibiting a significant hemostatic effect, while, furthermore, the solubilized carboxymethyl cellulose fibers have no effect whatsoever on the enzymes of the blood coagulation system such as coagulation factor XII.

Furthermore, the Japanese Patent Application No. Hei11-58412 refers to a soluble trauma-healing hemostatic cellulose fiber whose hydroxyl groups in the glucose units constituting the cellulose molecules of the natural or regenerated cellulose fibers have been partially

carboxymethylated so that its carboxymethyl substitution level (etherification degree) becomes 0.5 - under 1.0%.

The aforesaid soluble trauma-healing hemostatic cellulose fiber material does rapidly dissolve on contact with blood to exhibit a very potent hemostatic effect it has a blood-stilling effect only when coming in contact with blood. Its effect is thus due to its interactions with the platelets and fibrin of the blood so that in minor hemorrhage, that is to say, in loci (trauma sites) with little platelet and fibrin amounts it has a small hemostatic effect.

Summary of the Invention

In order to overcome the above difficulties, the inventors of the present application have conducted intensive investigations and found that soluble trauma-healing hemostatic cellulose fiber containing coagulation protein has a superior absorbency in tissue fluids, including blood, is readily dissolved on contact with blood, and even when the coagulation cascade is not active, it will exhibit a hemostatic effect due to stimulation of the agglutination reaction of fibrin monomer which is formed from fibrinogen by the action of the thrombin present in the coagulation protein-containing soluble trauma-healing hemostatic cellulose fiber and as a result of the stabilization of the agglutinates through a cross-linking reaction of the coagulation factor XIII that is contained in said soluble trauma-healing hemostatic cellulose fiber. In other words, the coagulation protein-containing soluble trauma-healing hemostatic cellulose fiber accelerates the agglutination of fibrin monomer formed through the action of thrombin regardless of the activation of the enzymes involved in the coagulation cascade reaction and, furthermore, promotes the adhesion and agglutination of the platelets to the trauma locus due to the rapid dissolving thereof on contact with the blood or body fluids present in the trauma site. It has thus been found to promote the adhesion activity of the fibronectin cells.

Thus, the invention according to this application refers to a material obtained by the application of coagulation proteins to a natural or regenerated cellulose fiber whose hydroxyl groups in the glucose units constituting the cellulose molecule have been partially carboxymethylated in such a manner that the carboxymethyl substitution level (etherification degree) becomes 0.5 - under 1.0%, and relates to a material consisting of a coagulation protein-containing soluble trauma-healing hemostatic cellulose fiber obtained by a process in which, after treatment of the natural or regenerated cellulose fiber in sodium hydroxide, the hydroxyl groups of the glucose units constituting the cellulose molecule are partially carboxymethylated by allowing said fiber to react with a monochloroacetic acid for a certain reaction time, preferably 4 - 18 hours, in such as manner that the substitution level (etherification degree) becomes 0.5 - under 1,0% with subsequent purification and in which, furthermore, the refined product is imparted with fibrinogen, thrombin and coagulation factor XIII as the coagulation proteins with subsequent drying.

The coagulation proteins are imparted by way of application to the aforesaid carboxymethylated natural or regenerated cellulose fiber or by way of chemical boding to the aforesaid carboxymethylated natural or regenerated cellulose fiber (that is to say, a means other than the physical bonding brought about by surface application).

Moreover, surface application of the coagulation proteins may be accomplished in practice, by way of example, by spraying a solution containing said coagulation proteins on to the aforesaid carboxymethylated natural or regenerated cellulose fiber. In this event, the coagulation proteins may be applied singly in a combination consisting of all of the three proteins referred to herein above, namely, fibrinogen, thrombin and coagulation factor XIII, or individually in successive order by first applying fibrinogen, then thrombin and finally the coagulation factor XIII.

In contrast, chemical bonding of the coagulation proteins may be accomplished, by way of example, by chemical reaction involving the addition of a solution containing said coagulation proteins to the aforesaid carboxymethylated natural or regenerated cellulose fiber which has been treated with a carbodiimide reagent. In this event, the coagulation proteins may be added for chemical reaction singly in a combination consisting of all of the three proteins referred to herein above, namely, fibrinogen, thrombin and coagulation factor XIII, or individually in successive order by first adding for chemical bonding fibrinogen, then thrombin and finally the coagulation factor XIII.

Furthermore the invention according to the present application refers to a product obtained by pulverizing the aforesaid carboxymethylated natural or regenerated cellulose fiber imparted with the coagulation proteins as described herein above after imparting said coagulation proteins thereto and after drying. Said powder may be obtained by pulverizing the aforesaid natural or regenerated cellulose fiber imparted with the three coagulation proteins, namely, fibrinogen, thrombin and coagulation factor XIII, in combination by single application or chemical bonding or, alternatively, by pulverizing the aforesaid natural or regenerated cellulose fiber imparted with the three coagulation proteins, namely, fibrinogen, thrombin and coagulation factor XIII, in successive order by consecutive application or chemical bonding with the subsequent mixing thereof.

Moreover, in accordance with the invention of this application, the aforesaid natural or regenerated cellulose fiber imparted with the three coagulation proteins is a drawn thread array consisting of a number of single threads loosely twisted together or a woven fabric produced either by plain-weaving or twill-weaving a drawn thread array consisting of a number of single threads loosely twisted together, whereby the thickness of the drawn thread array corresponds to a Denier number comprised between 20 - 100.

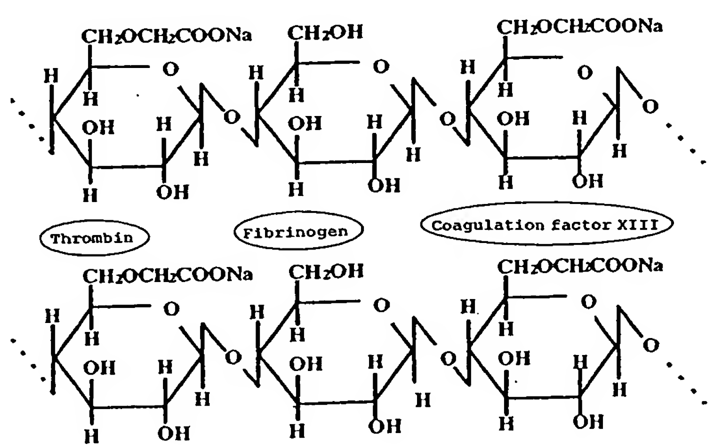
Furthermore, the invention according to the present application relates to a gauze-like product obtained by converting the aforesaid natural or regenerated cellulose fiber to a wool either before or after imparting the coagulation proteins thereto.

Furthermore, the invention according to the present application relates to product obtained by pulverizing, after imparting said coagulation proteins, the aforesaid natural or regenerated fibers in the form of a drawn thread area or a woven fabric or a gauze.

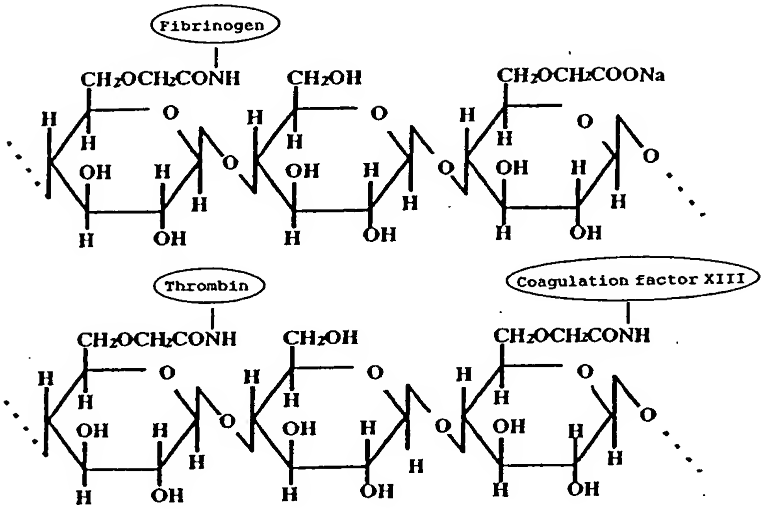
Moreover, the invention according to this application relates to a product consisting of coagulation protein-containing soluble trauma-healing blood-stilling cellulose fiber prepared in the manner afore-described with an enhanced hemostatic healing effect when applied to the trauma site.

Moreover, the preferred coagulation protein-containing soluble trauma-healing blood-stilling cellulose fiber in accordance with the invention of the present application may be represented by the following summary Chemical formula 1 or Chemical formula 2 as the structural units constituting the cellulose. Chemical formula 1 represents the arrangement in which the coagulation proteins have been imparted by surface application and Chemical formula 2 an arrangement in which the coagulation proteins have been imparted by chemical bonding.

[Chemical formula 1]



[Chemical formula 2]



Brief Description of the Drawings

Fig. 1 shows the relationship between absorption and time obtained by measuring the absorption indicating the effect of the soluble trauma-healing hemostatic cellulose containing coagulation protein prepared by the two methods being application and chemical bonding methods with respect to the fibrimonomer coagulation reaction.

Fig. 2 shows in (A) the coagulation rate/time dependence obtained by measuring the platelet coagulation condition in the absence of soluble trauma-healing hemostatic cellulose fiber, in (B) the coagulation rate/time dependence obtained by measuring the platelet coagulation condition in the presence of soluble trauma-healing hemostatic cellulose fiber, in (C) the coagulation rate/time dependence obtained by measuring the platelet coagulation condition in the absence of soluble trauma-healing hemostatic cellulose fiber containing coagulation protein prepared by the application method, and in (D) the coagulation rate/time dependence obtained by measuring the platelet coagulation condition in the absence of soluble trauma-healing hemostatic cellulose fiber containing coagulation protein prepared by the chemical bonding method.

Description of the Preferred Embodiments

The following descriptions use embodiments of the practical preparation of the soluble trauma-healing

hemostatic cellulose containing coagulation protein and examples of tests for achieving the trauma-healing and hemostatic effects.

As an example for the execution of the preparation of the soluble trauma-healing hemostatic cellulose, the Japanese Patent Application No. Heill- 58412 describes a method for the preparation of soluble trauma-healing hemostatic cellulose whereby 70g of natural or regenerated cellulose fiber formed into a fabric obtained by drawing an array of 20 loosely twisted fibers and by one-two twill-weaving the drawn fiber array with a thickness of 40 Denier, was introduced into a 1000mL rotary reaction vessel to which were added 250mL of sodium hydroxide - ethanol solution consisting of 38 volume parts of a 45% aqueous sodium hydroxide solution and 62 volume parts of 95% ethanol, with subsequent thorough impregnation and stirring for 2 hours at 25 degrees C. Following this, addition was made to this reaction solution of 210mL of a monochloro acetic acid reaction solution consisting of 40 volume parts of monochloro acetic acid and 60 volume parts of 95% ethanol, with subsequent stirring for 4 - 18 hours. After the reaction had reached completion, the hydrogen ion index (pH value) of the solution containing the fibers thus obtained was adjusted to 7.0 with 20% hydrochloric acid, whereupon the fibers were washed with a 60 - 95% aqueous ethanol solution until the sodium chloride content of the fibers we 1% or less. The cellulose fiber treated in this manner was then dried and sterilized to obtain a soluble trauma-healing hemostatic cellulose with the target etherification degree (level of carboxymethyl group substitution) of 0.5 - 1.0%. The "soluble trauma-healing hemostatic cellulose fibers" mentioned in the test examples below shall be interpreted as the soluble trauma-healing hemostatic cellulose fibers obtained by the afore-described means, unless where specifically stated otherwise.

Next, in test example 1, the etherification degree corresponding to the stirring time with the monochloro acetic acid reaction solution was determined in order to ascertain that the degree of etherification (carboxymethyl

group substitution) of the soluble trauma-healing hemostatic cellulose fibers thus obtained was within the range of 0.5 - 1.0%. The measurement method was such that 1g of each of the soluble trauma-healing hemostatic cellulose fibers produced by stirring with the monochloro acetic acid solution 2, 4, 8, 14, and 18 hours, respectively, in the aforesaid test example was cut into fine pieces, transferred into a tapering triangular (50mL) flask, added with 25mL of a nitric aid-methanol solution (using a solution prepared by mixing 100mL of methanol and 10mL of nitric acid), shaken for 1 hour and used as the hydrogen type sample. Following this, the sample was trapped by absorptive filtration in a glass filter (G3), washed with 120mL (three times using 40mL each time) with an 800g/L aqueous methanol solution (mixture of 100mL of anhydrous methanol and 20mL of water), and finally washed with 25mL of anhydrous methanol, whereupon the sample was passed through an absorption filter and the sample on the filter was dried at 105 degrees C for 2 hours. Furthermore, 0.2g of the hydrogen type sample was accurately weighed, placed in a (100mL) tapering triangular flask, added with 8mL of 800g/L methanol and 20mL of a standard 0.1mol/L sodium hydroxide solution and shaken for 30 minutes at 25 degrees C to convert the hydrogen type sample to the sodium type. Moreover, the excess sodium hydroxide was then determined by titration with a sulfuric acid of a known titer of 0.05mol/L using phenolphthalein as the indicator in order to determine the etherification degree therefrom. Table 1 below presented the measurement results.

[Table 1]

Denier number of soluble trauma-healing hemostatic cellulose fibers	Degree of etherification (Carboxymethyl Group Substitution)				
	Stirring Reaction Time (hr)				
	2	4	8	14	18
1	0.410	0.612	0.701	0.801	0.856
2	0.401	0.611	0.693	0.793	0.823
3	0.421	0.632	0.721	0.812	0.842
4	0.425	0.625	0.688	0.801	0.825
5	0.416	0.601	0.701	0.812	0.831

As can be seen from the results presented in Table 1 herein above, when the reaction time with monochloro acetic

acid is four hours or more it is possible to produce a soluble trauma-healing hemostatic cellulose having a degree of substitution of 0.5% or more. Consequently, it is possible to control the degree of carboxymethyl group substitution by controlling the reaction time with monochloro acetic acid.

Next, in test example 2, the method of preparation of soluble trauma-healing hemostatic cellulose fibers containing coagulation protein is explained, being a method for the application of coagulation protein to soluble trauma-healing hemostatic cellulose fibers and natural or regenerated cellulose fibers. There are two different methods of imparting coagulation protein, one of which is the application and the other one the chemical bonding method, and each will be explained herein below.

First, while the soluble trauma-healing hemostatic cellulose fibers are soluble in water, in the case of an aqueous solution containing 60% of ethanol or more the fiber will not dissolve and can thus be preserved as fiber so that the application method may consist of a procedure by which a soluble trauma-healing hemostatic cellulose fiber containing coagulation protein is obtained by application in such a manner that 0.2mL of a 60% ethanol solution containing, as coagulation proteins, 5mg of fibrinogen, 1.5 units of thrombine, and 8 units of blood coagulation factor XIII, is uniformly sprayed on to 15.6mg ($1cm^2$) of soluble trauma-healing hemostatic cellulose fiber prepared in accorance with the embodiment described above (with a reaction time of 14 hours with monochloro acetic acid) while allowing to dry with a fan.

Conversely, the chemical bonding method takes place by a procedure involving the use of carbodiimide in such a manner that 15.6mg of the soluble trauma-healing hemostatic cellulose fiber (fiber obtained by reaction with monochloroacetic acid for 14 hours) produced in accordance with the afore-described embodiment is introduced into a 5mL capacity glass test tube and after addition of 1mL of a 60% ethanol solution, 38mg of 1-ethyl-3-(3-dimethyl amino propyl) carbodiimide is added with subsequently stirring

at 30 degrees C for 2 hours, whereupon the reaction solution is removed and the soluble trauma-healing hemostatic cellulose fiber treated with said carbodiimide is washed three times with 3mL of a 60% ethanol solution. Next, addition is made thereto of 1mL of a 60% ethanol solution containing, as coagulation proteins, 5mg of fibrinogen, 1.5 units of thrombine, and 8 units of blood coagulation factor XIII for reaction at 30 degrees C for 2 hours. In order to block the unreacted carboxyl groups, addition is made of 29mg of L-lysine and after allowing to react at 30 degrees C for 1 hours, the reaction solution is removed and the post-reaction soluble trauma-healing hemostatic cellulose fiber is washed three times with 3mL of a 60% ethanol solution and once with 3mL of a 95% ethanol solution. Following this, said soluble trauma-healing hemostatic cellulose fiber is dried at 50 degrees C for five minutes to obtained a soluble trauma-healing hemostatic cellulose fiber with chemically bonded coagulation protein.

Following this, the solubility of the coagulation protein-containing soluble trauma-healing hemostatic cellulose fiber in a 0.95% sodium chloride (salt) solution and in pure water is measured as a third test example in order to ascertain the solubility of the coagulation protein-containing soluble trauma-healing hemostatic cellulose fiber obtained by either of the aforesaid method, namely, surface application and chemical bonding. The method by which these measurements are carried out is to add 0.1g of the coagulation protein-containing soluble trauma-healing hemostatic cellulose fiber obtained by either of said methods 100mL of an 0.95% aqueous salt solution and an equal volume of pure water, respectively, (so that the concentration of said fiber becomes 0.1w/v%) and measure by visual inspection the time required until the insoluble parts have completely disappear while continuing to stir at 25 degrees C. The results are reported in Table 2.

[Table 2]

Adjustment Method	Denier No. of coagulation protein-containing soluble trauma-healing hemostatic cellulose fiber	Time required until completely dissolved (minutes)	
		Pure water	0.95 saline solution
Surface application	1	10	18
	2	11	19
	3	9	17
	4	12	17
	5	10	19
Chemical bonding	1	9	19
	2	11	19
	3	10	18
	4	9	17
	5	11	19

As can be seen from the results presented in Table 2 herein above, coagulation protein-containing soluble trauma-healing hemostatic cellulose fiber in accordance with the invention of this Application is readily and completely soluble, without fail, in pure water and in saline water regardless of whether said coagulation protein is imparted by way of surface application or by chemical bonding methods.

Given as a fourth test example is the measurement of the 350nm absorption degree using an ultraviolet spectrophotometer U-3210 (manufactured by Hitachi,Ltd.) in order to ascertain the fibrinomonomer coagulation activity of the coagulation protein-containing soluble trauma-healing hemostatic cellulose fiber obtained by either of the aforesaid method, namely, surface application and chemical bonding. For the measurement conducted in the presence of the 1w/v% coagulation protein-containing soluble trauma-healing hemostatic cellulose fiber obtained by either of the aforesaid method (namely, surface application and chemical bonding), 20 μL of fibrinomonomer (A280nm = 6) dissolved in 20mmol/L of acetic acid was added to 500 μL of 20mmol/L imidazol buffer solution (pH7.4) containing 0.15mol/L of sodium chloride (NaCl) in the presence of the soluble trauma-healing hemostatic cellulose fiber (that is, soluble trauma-healing hemostatic cellulose fiber obtained without imparting

coagulation protein and in the absence of soluble trauma-healing hemostatic cellulose fiber (control), and 20 seconds after of fibrinomer 350nm absorptivity was measured every 30 seconds for 25 minutes. The results of these measurements are reported in Fig. 1.

As can be seen from the results assembled in Fig. 1, the coagulation protein-containing soluble trauma-healing hemostatic cellulose fiber is capable of substantially promoting the coagulation of fibrinomer, regardless of whether said coagulation protein is imparted by way of surface application or by chemical bonding methods, and it can be recognized furthermore, that the fibrinomer coagulation activity is much superior as compared with a soluble trauma-healing hemostatic cellulose fiber to which no coagulation protein has been imparted.

Next, in test example 5, the platelet agglutination ability has been measured using a platelet agglutination test unit (manufactured by Mebanics) in order to ascertain the platelet agglutination promoting activity of the coagulation protein-containing soluble trauma-healing hemostatic cellulose fiber obtained by either of the aforesaid methods, namely, surface application and chemical bonding, and to establish the significantly superior platelet agglutination promoting activity of the aforesaid coagulation protein-containing soluble trauma-healing hemostatic cellulose fiber as compared with the soluble trauma-healing hemostatic cellulose fiber to which no coagulation protein has been imparted. For the measurement of the platelet agglutination ability, 20mmol/L imidazol buffer solution containing 0.15mol/L of sodium chloride (NaCl) in the presence of the soluble trauma-healing hemostatic cellulose fiber (that is, soluble trauma-healing hemostatic cellulose fiber obtained without imparting coagulation protein and in the absence of soluble trauma-healing hemostatic cellulose fiber, was mixed with platelet-rich plasma at a ratio of 1 : 1 and adenosine diphosphate (ADP) was added at concentrations corresponding to its function as a platelet agglutination agent to determine the platelet agglutination ability by measuring the turbidity of the reaction solution. The

results of the measurements are presented in Table 3 and Fig. 2.

[Table 3]

	Channel No.	ADP concentration (μmol/L)	Max. agglutination rate (%)	Expression time (min)	5.0 minute value of agglut-ination (%)	5.0 minute area	Separation ratew (%)
In the absence of soluble trauma-healing hemostatic cellulose fiber	1	0.5	63	1.9	52	2795	38
	2	1.0	62	2.7	57	2783	20
	3	2.0	66	2.7	66	2973	10
	4	4.0	65	3.7	65	2839	3
In the presence of soluble trauma-healing hemostatic cellulose fiber	5	0.5	65	3.7	64	2910	8
	6	1.0	68	6.1	68	2943	0
	7	2.0	69	6.5	68	2929	0
	8	4.0	68	6.5	68	2929	0
In the absence of soluble trauma-healing hemostatic cellulose fiber containing coagulation protein prepared by surface application	9	0.5	99	10	95	2910	0
	10	1.0	97	9.5	96	2950	0
	11	2.0	94	9.8	98	2960	0
	12	4.0	95	9.7	92	2930	0
In the presence of soluble trauma-healing hemostatic cellulose fiber containing coagulation protein prepared by chemical bonding	13	0.5	95	9.9	94	2940	0
	14	1.0	96	9.7	94	2950	0
	15	2.0	99	9.9	97	2940	0
	16	4.0	98	9.8	95	2970	0

As can be seen from the results presented in Table 3 and Fig. 2, the coagulation protein-containing soluble trauma-healing hemostatic cellulose fiber is capable of substantially promoting platelet agglutination, regardless of whether said coagulation protein is imparted by way of surface application or by chemical bonding methods, and it can be recognized furthermore, that the platelet agglutination activity is much superior as compared with a soluble trauma-healing hemostatic cellulose fiber to which no coagulation protein has been imparted.

Next, in test example 6, the cell adhesion ability has been measured by determining the adhered cell count in a given field of view in order to ascertain the cell adhesion promoting activity of the coagulation protein-containing soluble trauma-healing hemostatic cellulose fiber obtained by either of the aforesaid methods, namely, surface application and chemical bonding, and to establish the cell adhesion promoting activity of the aforesaid coagulation protein-containing soluble trauma-healing hemostatic cellulose fiber and to demonstrate the superior cell adhesion ability thereof as compared with the soluble trauma-healing hemostatic cellulose fiber to which no coagulation protein has been imparted. For this purpose, the adhered cell count upon addition of the respective soluble trauma-healing hemostatic cellulose fiber containing 1w/v% coagulation protein imparted by either of said methods, and the adhered cell count upon addition of soluble trauma-healing hemostatic cellulose fiber (that is, soluble trauma-healing hemostatic cellulose fiber not containing coagulation protein), and the adhered cell count without addition of soluble trauma-healing hemostatic cellulose fiber were determined. The measurement of the adhered cell count was made in such a manner that 96 well plates were covered with the cell adhesion proteins fibrinectin, vitronectin, laminin, collagen and fibrin, respectively, at the various concentrations, and that 5,000 NIH-3T3 were then introduced thereon to determine the respective adhesion protein concentrations at which no cell adhesion activity is in evidence by determining the given adhered cell count in a given field of view after six hours and by determining the adhered cell count in a given field of view following the addition of 1w/v% coagulation protein-containing soluble trauma-healing hemostatic cellulose fiber obtained by either of the aforesaid method or soluble trauma-healing hemostatic cellulose fiber, respectively, and, alternatively, without any addition whatsoever. The results of these measurements are reported in Table 4.

[Table 4]

Cell adhesion protein	Concentration of cell adhesion protein (ug/mol)	Adhered cell count			
		Without addition of soluble trauma-healing hemostatic cellulose fiber	With addition of soluble trauma-healing hemostatic cellulose fiber	With addition of soluble trauma-healing hemostatic cellulose fiber containing coagulation protein prepared by surface application	With addition of soluble trauma-healing hemostatic cellulose fiber containing coagulation protein prepared by chemical bonding
Fibronectin	0.1	20	80	320	313
Vvitronectin	0.5	10	60	295	290
Laminin	0.1	12	56	290	300
Collagen I	0.1	5	60	289	285
Collagen III	0.1	8	62	292	296
Fibrin	0.1	10	60	290	299

As can be seen from the results presented in Table 4, the coagulation protein-containing soluble trauma-healing hemostatic cellulose fiber exhibits a substantially higher adhered cell count, regardless of whether said coagulation protein is imparted by way of surface application or by chemical bonding methods, and it can thus be recognized that said coagulation protein-containing soluble trauma-healing hemostatic cellulose fiber possess a cell adhesion promoting activity, and that, furthermore, the cell adhesion promoting activity of said coagulation protein-containing soluble trauma-healing hemostatic cellulose fiber is significantly superior to that of the soluble trauma-healing hemostatic cellulose fiber to which no coagulation protein has been imparted.

Next, in test example 7, measurements have been conducted in otder to determine the blood-stilling or hemostatic effect and the trauma-healing effect by using coagulation protein-containing soluble trauma-healing hemostatic cellulose fiber obtained by either of the aforesaid methods, namely, surface application and chemical bonding, on the trauma site, and in order to establish the substantially superior blood-stilling or

hemostatic effect and the trauma-healing effect thereof as compared with the soluble trauma-healing hemostatic cellulose fiber to which no coagulation protein has been imparted. For this purpose, the time required for hemostasis to occur upon administration to the trauma site of the respective soluble trauma-healing hemostatic cellulose fiber containing 1w/v% coagulation protein imparted by either of said methods and the extent of healing of the trauma, the time required for hemostasis to occur upon administration to the trauma site of the soluble trauma-healing hemostatic cellulose fiber not containing coagulation protein and the extent of healing of the trauma site, and the time required for hemostasis to occur without administration to the trauma site of the respective soluble trauma-healing hemostatic cellulose fiber and the extent of healing of the trauma site were determined. The hemostasis time was determined by resecting 1cm x 1cm square patches of liver from 10 rats and attaching to the trauma site coagulation protein-containing soluble trauma-healing hemostatic cellulose fiber and soluble trauma-healing hemostatic cellulose fiber, respectively, and attaching no soluble trauma-healing hemostatic cellulose fiber (whatsoever), respectively, to measure the time required for hemostasis to occur (seconds), whereby a comparison was made with the hemostasis effect associated with the untreated trauma and that associated with the fiber to which no coagulation protein had been imparted; and, furthermore, the extent of healing was determined by attaching to the trauma site the respective soluble trauma-healing hemostatic cellulose fiber containing coagulation protein imparted by either of said methods, closing the abdominal section after the hemostatis time had been measured, performing laparotomy one month later and taking a pathology section of the trauma site for preparation and microscopic observation for visual assessment of the degree of healing. The results of the measurements are reported in Table 5. For the determination of the extent of healing of the trauma site, the specimens equivalent to the normal condition were marked with the ◎ sign, those exhibiting some slight inflammation with the ○ sign and those showing complete healing of the inflammatory processes with the × sign.

[Table 5]

Rats No.	1	2	3	4	5	6	7	8	9	10	Mean	CV
Hemostasis time (secs.) without treatment using soluble trauma-healing hemostatic cellulose fiber	152	141	180	160	120	150	120	137	165	201	153	16.7%
Hemostasis time (secs.) with treatment using soluble trauma-healing hemostatic cellulose fiber	29	32	31	37	34	35	32	27	35	35	33	9.5%
	◎	◎	◎	◎	◎	◎	◎	◎	◎	◎		
Hemostasis time (secs.) with treatment using hemostatic cellulose fiber containing coagulation protein prepared by surface application	10	12	13	11	8	9	12	11	10	9	11	15.1%
	◎	◎	◎	◎	◎	◎	◎	◎	◎	◎		
Hemostasis time (secs.) with treatment using hemostatic cellulose fiber containing coagulation protein prepared by chemical bonding	11	10	12	9	12	11	8	9	10	12	10	13.7%
	◎	◎	◎	◎	◎	◎	◎	◎	◎	◎		

As can be seen from the results presented in Table 5, soluble trauma-healing hemostatic cellulose fiber has a substantial blood-stilling or hemostatic effect, and the ten rats treated with soluble trauma-healing hemostatic cellulose fiber exhibited a virtually complete healing without any signs of inflammation whatsoever, and it can therefore be recognized that coagulation protein-containing soluble trauma-healing hemostatic cellulose fiber possess a substantial hemostatic and trauma-healing effect as compared with soluble trauma-healing hemostatic cellulose, regardless of whether said coagulation protein is imparted by way of surface application or by chemical bonding methods.

The coagulation protein-containing soluble trauma-healing hemostatic cellulose fiber produced as described herein above and in accordance with the invention of this Application absorbs both blood and tissue fluid when applied to the trauma site, regardless of whether said coagulation protein is imparted by way of surface application or by chemical bonding methods, increases the concentration and viscosity of the blood and the tissue fluid, and generates potent fibrin agglutinates under the action of the fibrinogen, thrombin and coagulation factor XIII contained in said coagulation protein-containing soluble trauma-healing hemostatic cellulose fiber, thereby exhibiting a powerful blood-stilling or hemostatic effect in such as manner as to reduce the flow (rate) of the blood

and tissue fluid; and exihiting, furthermore, a blood-stilling or hemostatic effect by assisting platelet adhesion to , and platelet agglutination in, the trauma site.

Moreover, coagulation protein-containing soluble trauma-healing hemostatic cellulose fiber in accordance with the inventions of the present Application interacts with the adhesion proteins such as fibronectin and promotes the growth of fibroblasts which play an important role in the trauma healing process.

While the explanations concerning the test examples described herein above refer to the sodium salt of the coagulation protein-containing soluble trauma-healing hemostatic cellulose fiber, the invention according to the present Application is not limited thereto but may, without any limitation whatsoever, also refer to the calcium salt of the coagulation protein-containing soluble trauma-healing hemostatic cellulose fiber or to mixtures of a multiplicity of salts thereof.

Whereas, furthermore, all of the explanations concerning the test examples described herein above refer to a coagulation protein-containing soluble trauma-healing hemostatic cellulose fiber formed into a fabric the invention according to the present Application is not limited thereto but may naturally also apply to a product formed into a powder by subjecting the threadlike or fabric-like coagulation protein-containing soluble trauma-healing hemostatic cellulose fiber to a pulverization treatment or to a product formed into a gauze material by subjecting the threadlike or fabric-like coagulation protein-containing soluble trauma-healing hemostatic cellulose fiber to a shoddy wool treatment, provided that any such coagulation protein-containing soluble trauma-healing hemostatic cellulose fiber product does exhibit a trauma-healing and hemostatic effect as being the purpose of the present invention.

As explained herein above, the coagulation protein-containing soluble trauma-healing hemostatic

cellulose fiber in accordance with the invention of the present Application has an extremely speedy blood-stilling or hemostatic effect, presents a speedy in-vivo absorption behavior virtually without giving rise to any inflammatory reactions and exhibits a high trauma-healing effect.

Furthermore, the coagulation protein containing soluble trauma-healing and hemostatic material in accordance with the invention of the present Application is capable of producing a trauma-healing effect based on its ability to promote hemostasis and cell adhesion in the trauma site at high efficiency; wherefore it is eminently effective a a hemostatic and trauma-healing material for both internal and external application and has a significant scope for potential application in a wide range of uses as a trauma-covering hemostatic material capable of enhancing the healing effect of the trauma locus.